MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD - MAPPING

Contract # N01-NS-5-2332

First Progress Report
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Neural Prosthesis Program

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I. Introduction

During this quarter progress has been made in three areas: (1) Recording of pressure changes in the corpus cavernosous of the cat penis to stimulation of the S_1 ventral roots and microstimulation of the S_1 spinal cord. The changes in corpus cavernosous pressure seems to be a good method of quantifying the erectile response of the penis to microstimulation. (2) Studies continued during this quarter to determine the location and distribution in the spinal cord of efferent, afferent and interneurons which innervate the bladder, penis, or external urethral sphincter. (3) The design and construction of hardware to hold the cat spinal cord rigid while recording torque and position information form the hindlimb to activation of the flexor and extensors of the knee joint began during this quarter. In addition computer hardware has been assembled and software written to collect data from a variety of sensors during hindlimb motor experiments, as well as bladder, sphincter, and penile experiments.

II. Modulation of Penile Activity in Both the Anesthetized and Unanesthetized Decerebrate Animal

One of the major areas of investigation during this quarter was to determine the best preparation to produce penile erection in the cat to ventral root and spinal cord stimulation. Our early studies seemed to indicate that ∝-chloralose, although an excellent anesthetic for studying bladder reflexes in the cat was not very promising for studying penile erection or external urethral sphincter activity . Neither ventral root or cord stimulation in the ∝-chloralose anesthetized cat could produce penile protrusion or changes in corpus cavernosous pressure.

Preliminary results obtained from decerebrate unanesthetized animal preparations indicated that penile protrusion could easily be produced to ventral root stimulation. In subsequent experiments it was determined that pentobarbital (15 - 20mg/kg. i.v.) anesthesia in either decerebrate or intact cats did not interfere with penile protrusion produced by either ventral root or cord stimulation; in fact pentobarbital may actually improve the responses seen in the decerebrate animal. This suggests that some inhibitory mechanisms are present in the decerebrated animal which reduce penile protrusion in the cat. This may possibly be via the sympathetic nervous system which is known to modulate penile erection.

Although measurement were made of changes in penile length in two animals (30 - 40% increase in length to 30 - 60 sec stimulation of the S_1 root), a better and more accurate method of quantifying penile erection was needed. Pressure changes in the corpus cavernosous had been used previously in rat and cat experiments. We found that this was a reliable method, but position of the recording needle within the cavernous sinus was critical for stable pressure recordings.

The methods used for penile experiments were similar to those used in bladder and EUS experiments except for the following. The animals were either decerebrate or intact and pentobarbital anesthesia was used. The decerebration was a precollicular with complete removal of the forebrain and hypothalamus rostral to the superior colliculus (See Figure 1). A cannula via the bladder dome was used to record intravesical pressure, while a 25 gauge hypodermic needle or a 22 gauge intracath filled with heparin and positioned in the corpus cavernosous was used to record penile pressure (See Figure 2). Each catheter was connected to a pressure transducer and output displayed on a chart recorder and recorded on magnetic tape. Although pentobarbital anesthesia reduced or abolished rhythmic bladder contraction, stimulus induced contractions

were present and changes in penile pressure were easily produced by ventral root stimulation.

The stimulus parameters to produce penile pressure changes to either ventral root or spinal cord stimulation were different form those necessary to produce bladder contraction. Penile erection was produced best at frequencies of 25 - 35 Hz with a long duration of stimulation - 20 - 60sec; while good bladder contractions could be produced at 15 - 20 Hz at 10 sec duration of stimulation. The intensities of stimulation of ventral roots with hook electrodes for both bladder and penile responses are similar (0.5 - 2 volts) but intensities are slightly higher for cord stimulation (100 - 150μA at 100μ sec pulse duration) with fine tipped (200 - 400 μ^2) electrodes. The latency to pressure rise was considerably longer for penis than for bladder. The latency for penis was often 10 - 30 secs where as with bladder it was usually less than 2 sec.

The best response for penile erection was seen on the S_1 ventral root with responses sometimes seen on the S_2 root as well. The best bladder responses however were seen with S_2 stimulation and at times S_3 stimulation. Figure 3 shows the response to both penis (top) and bladder (bottom) to S_1 ventral root stimulation. The left and right S_1 ventral roots are stimulated individually (L or R on Figure 3) and simultaneously (B on Figure 3). The early sharp and short lived response seen with root stimulation is due to striated muscle probably of the bulbocavernosus. A neuromuscular blocking agent (pavulon, 125 μg/kg, i.v.) blocks this response without effecting the slower smooth muscle response of the corpus cavernosous. In the experiment shown in Figure 3 both roots produce an almost equal response. This is not the case, however, in every experiment. Often one root gives a better response than the other and usually this root is ipsilateral to the cavernous sinus being recorder. In the experiment shown in Figure 3 some inhibition is seen when both roots are stimulated simultaneously. This type of interaction was not seen in every animal. These differences from animal to animal many relate to the

position of the recording needle. Notice also in Figure 3 (bottom) that the root which gave the best penile response gave only a small response on the bladder with S_1 stimulation.

Figure 4 shows the response to caudal S_1 spinal cord microstimulation (100μA, 0.1 msec duration, 30 Hz for 60 sec) with a fine tipped (400 μ^2) activated iridium microelectrode. The response was seen only over a distance of 400 or 500 μ and at a depth of 1.8 - 2.0 mm form the cord surface. This corresponded to the area of the sacral parasympathetic nucleus. Although our mapping studies of the penile responses in the spinal cord, are preliminary, in general good responses seem to require higher intensities (at least 100 μA) of stimulation compared to bladder and these responses are often seen in a more restricted area of cord.

These types of studies will continue into the next quarter, as we try to map these responses in various areas of the spinal cord.

III. Penile, Bladder and External Urethral Sphincter Neurons Revealed by Pseudorabies Virus Tracing Techniques

The pseudorabies virus tracing studies described in previous progress reports continued during this quarter and are summarized below.

These studies are designed to examine the location and distribution of efferent, afferent and interneurons from the penis, bladder and external urethral sphincter. Since PRV is a transynaptic tracer, interneurons as well as first order neurons innervating the injected organ are labeled. Data from these and other tracing experiments suggest possible sites for the selective modulation or activation of bladder, penile or sphincter responses with microstimulation as well as clues as to the interactions observed between these organ systems. For example we know that during normal micturition,

contraction of the bladder is accompanied by the relaxation of the external urethral sphincter (EUS). The location of first order efferent neurons for both bladder smooth muscle and EUS are known, but the interconnections that mediate the reciprocal interactions have not been established. PRV tracing has labeled interneurons which might mediate this interaction. Our studies suggest that the sacral parasympathetic preganglionic neurons to the bladder are connected to the motoneurons of the EUS. These connections may occur via a direct collateral connection or via an interneuron. Labeled interneurons in the dorsal commissure are seen following both bladder and EUS injections and focal microstimulation of these sites can modulate EUS activity, suggesting at least an interneuronal link between the sacral parasympathetics, mediating bladder contraction and the EUS motoneurons in Onuf's nucleus.

During this quarter we have extended some of our earlier studies by doing selective nerve cuts. In these studies the pudendal nerve, which provides input to the striated muscle of the EUS, were cut bilaterally and PRV injected into the bladder wall. These experiments eliminated the possibility that motoneurons in Onuf's nucleus are labeled by peripheral connections and implicate interneuronal or direct intraspinal connections.

During this quarter we extended our studies on the labeling of bladder neurons in the sacral cord to examine the possibility that some sacral cord labeling may be indirect via sympathetic neurons from the upper lumbar cord. In these experiments all the sacral roots (S_1, S_2 and S_3, both dorsal and ventral) were sectioned and the bladder wall was injected with PRV. Any sacral cord labeling must occur by an indirect pathway, possibly via the lumbar sympathetics. Little labeling was seen in the sacral cord suggesting that the prominent interaction between the sympathetic and parasympathetic bladder preganglionics occurs at some higher spinal or supraspinal site(s).

Further experiments utilizing selective nerve cuts will continue during the next quarter. In addition, studies tracing motoneurons and interneurons to the flexors and extensors of the hindlimb will begin.

IV. Progress in the Construction and Assembly of the Components Needed for Motor Studies of the Hindlimb.

During this quarter our electronic and machine shops have build new, and modified existing equipment to allow us to stimulate sites in the lumbar cord and record the torque and position from the knee joint of the cat hindlimb.

Modifications to the spinal frame were necessary to raise then animal thus allowing free access to and free movement of the hindlimb. Some additional clamps were added to the spinal frame to keep the lumbar cord rigid during leg movement and portioning. In addition, a leg holder with a knee joint pivot to hold the torque and angle sensors is under construction. The torque sensors were special ordered and took 12 weeks for delivery and this has delayed the construction of the holder.

The electronics and computer system to record and analyze movement data has been assembled and tested. A block diagram of the 1st phase of our studies is shown in figure 5. The system consists of a Pentium PC with a National Instrument data acquisition board running Lab View software. The system needs only to be connected to the signal conditioning amplifiers. Figure 6 shows a block diagram of our second phase of studies which will incorporate a torque motor and accelerometer which would allow us to determine compliance about the knee joint as well as establish other additional parameters of hindlimb movement.

The studies on the motor system should begin in the next quarter and our second phase experiments will begin later this year.

Figure 1 Schematic diagram of a midline sagittal section of the cat brain showing the level of decerebration (top figure - dotted line). The entire forebrain rostral to the superior colliculus is removed (bottom - shaded area). The level of decerebration is very important especially for the bladder experiments, since destruction of areas near the inferior colliculus will prevent bladder and EUS reflexes. For penile experiments the level of decerebration is probably less critical. SC = superior colliculus IC = inferior colliculus.

Figure 2 Schematic diagram of the experimental set-up for penile erection experiments in which pressure from the corpus cavernosous is recorded. In these experiments bladder pressure is recorded via a catheter placed in the dome of the bladder. The ureters are cut, ligated and drained externally. A 25 gauge hypodermic needle is placed in the cavernous sinus and connected to a pressure transducer. Changes in corpus cavernous pressure has correlated fairly closely with penile protrusion.

Figure 3 Chart recorder output showing penile pressure (top trace) recorded from the cavernosous sinus and bladder pressure (bottom trace) recorded via a catheter in the bladder dome to stimulation of the S_1 ventral roots. L = left ventral root stimulation, R = right ventral root stimulation, and B = both roots stimulated simultaneously. Stimulus parameters are 30 Hz, 50 μsec negative first bipolar pulses for 1 min at 2 volts. The stimuli are applied to the ventral roots at the black bars at the bottom of the figure. Notice the quite large changes in penile pressure with root stimulation. Both roots stimulated together seem to produce some inhibition. The fast very early response is a striated muscle response since it is blocked by pavulon a neuromuscular blocking agent (not shown). Notice that S_1 root stimulation gives a small bladder

response. The response seen from the S_2 root produced a large bladder contraction and small penile pressure change.

Figure 4 Chart recorder output showing penile pressure (top) and bladder pressure (bottom) to microstimulation of the S_1 segment of the spinal cord at different depths from the surface of the cord. The penetration begins near the dorsal root entry zone. The black bars at the bottom of the figure indicate the time of presentation of the stimulus to the spinal cord. The number below the stimulus bars are the depth in mm from the cord surface. The stimulus parameters are 30 Hz 200μsec duration, negative first bipolar pulses, for 1 min. This record is from the same animal as in Figure 3 with pressure recorded in the same manner. Notice the large penile pressure changes at a depth of 1.8 and 2.0 mm below the surface, while the bladder response is small at this location. The prediction would be that the response to S_2 spinal cord stimulation would be a large bladder and either no or a small penile response. S_2 was not examined in this particular animal.

Figure 5 A schematic diagram showing the experimental set-up for the motor system experiments which will examine the torque, position, and EMG of the knee flexor and extensor muscles to microstimulation of the spinal cord. A Pentium PC fitted with a data acquisition board will be used for data recording, display and analysis.

Figure 6 Same as Figure 5 except later in the contract period a torque motor with feedback control will be added to the system allowing compliance to be calculated and additional types of motor system experiments to be performed.

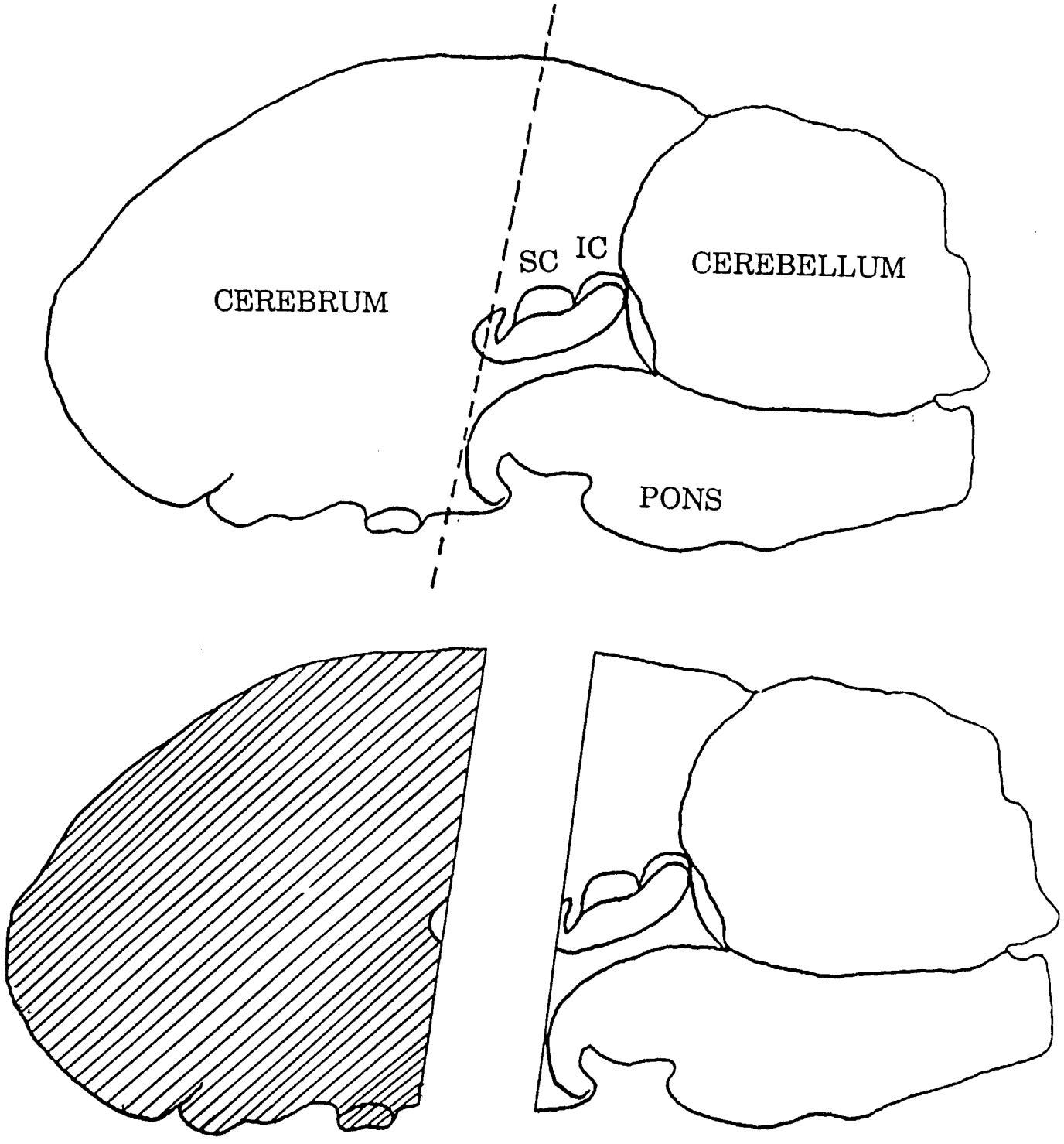


Figure 1

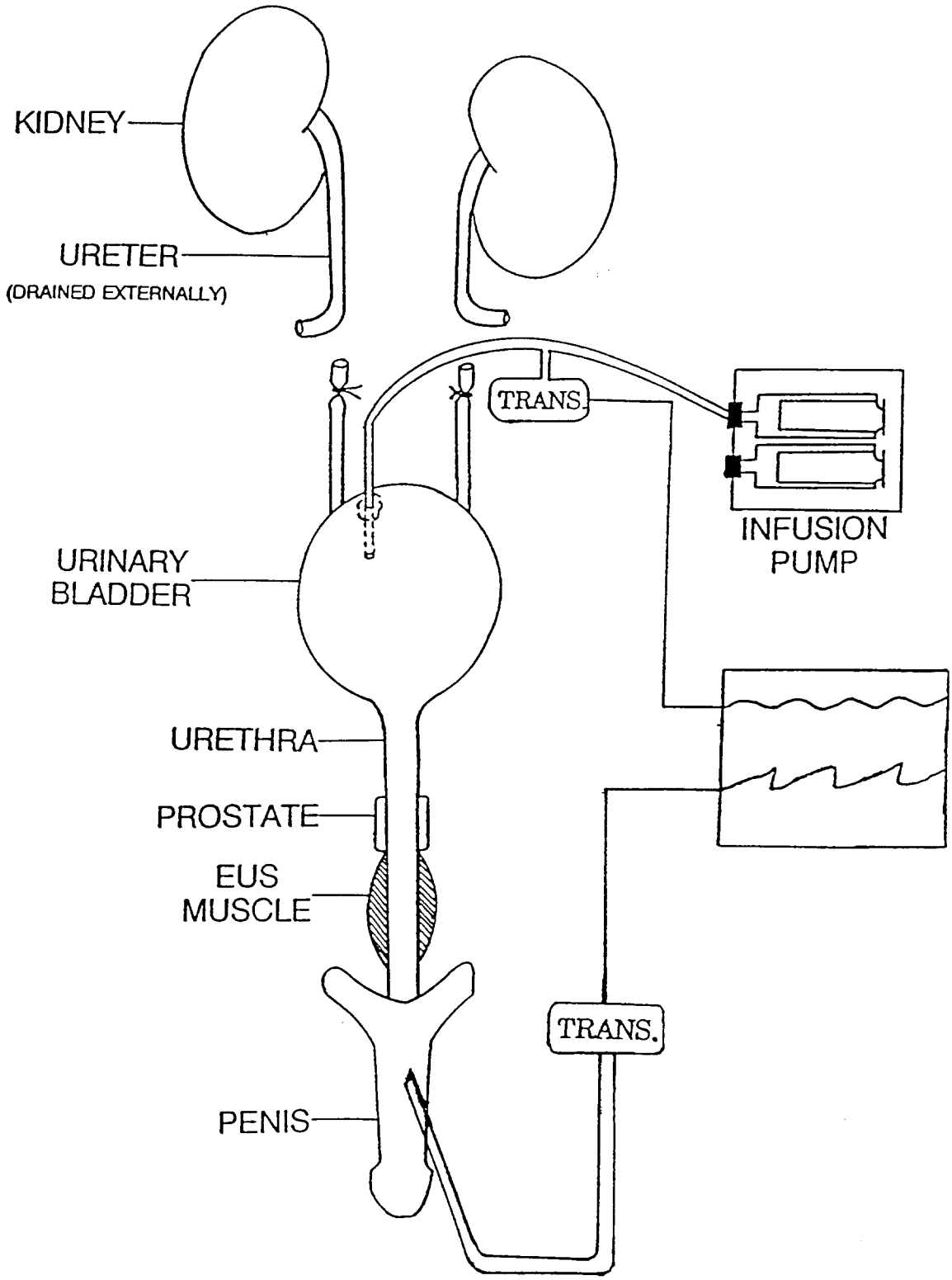

KIDNEY
URETER
(DRAINED EXTERNALLY)
TRANS.
INFUSION
PUMP
URINARY
BLADDER
URETHRA
PROSTATE
EUS
MUSCLE
TRANS.
PENIS

Figure 2

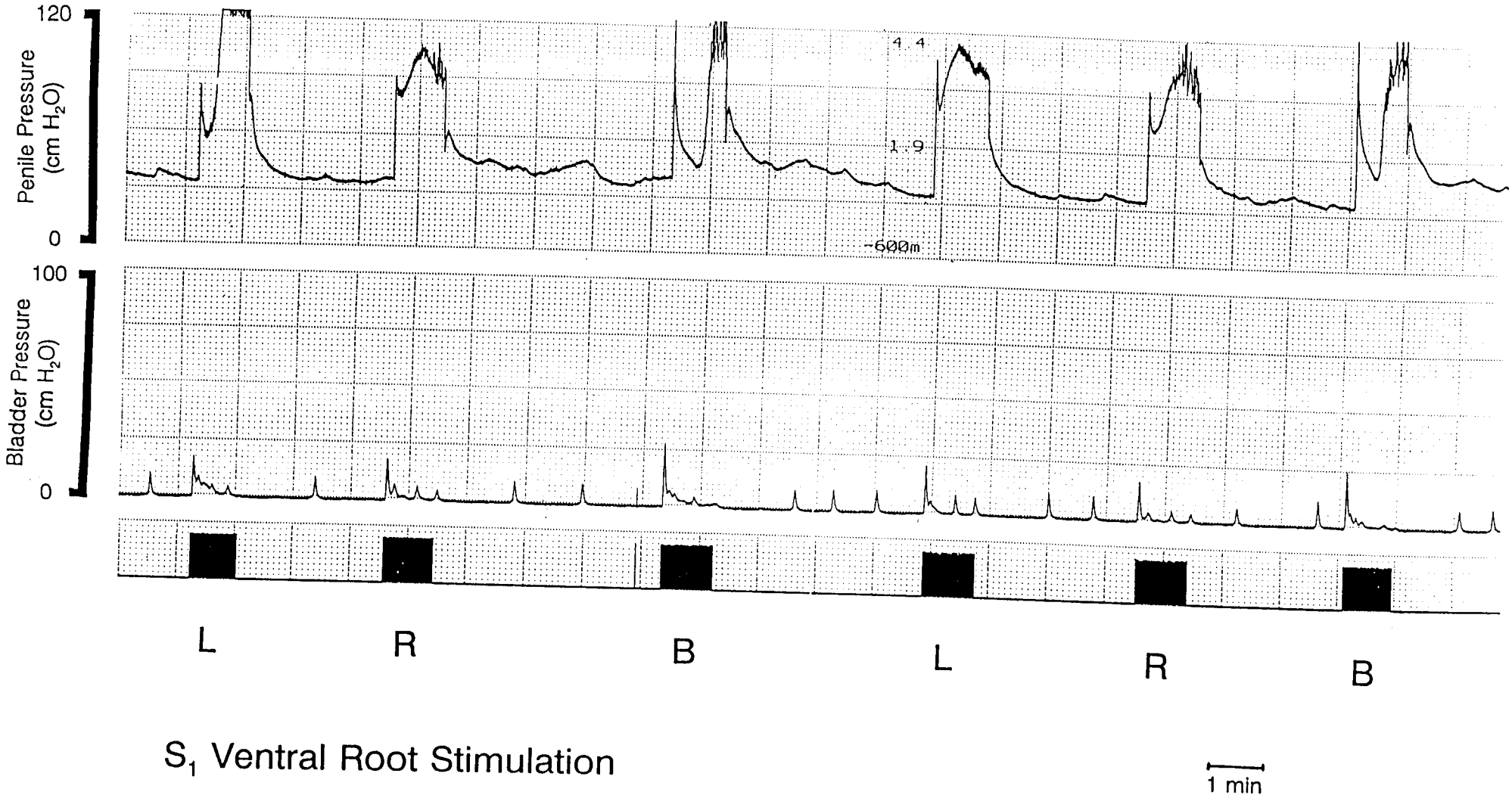
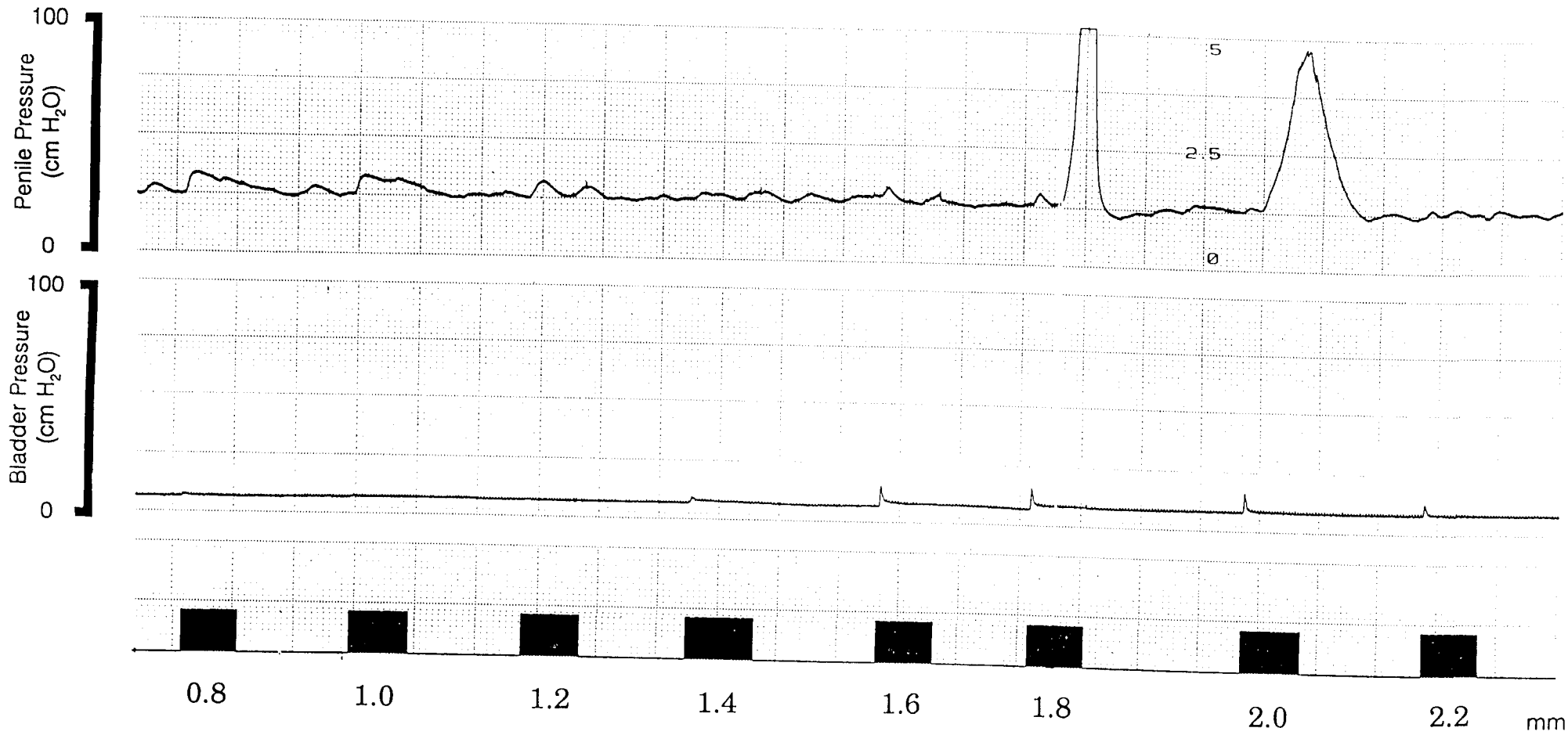

120
Penile Pressure
(cm H_2O)
0
4.4
1.9
~600m
100
Bladder Pressure
(cm H_2O)
0
L
R
B
L
R
B
S_1 Ventral Root Stimulation
1 min

Figure 3



S_1 Spinal Cord Stimulation

1 min

Figure 4

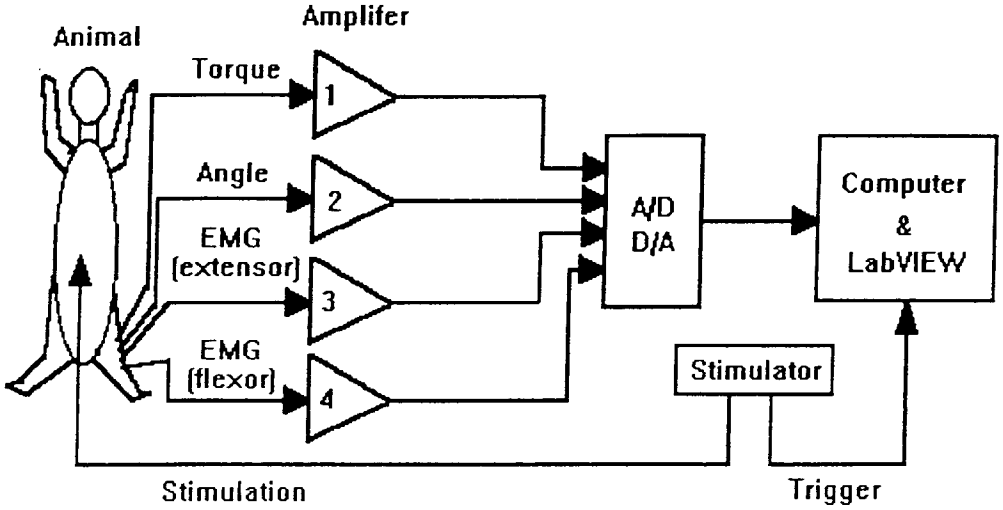


Figure 5

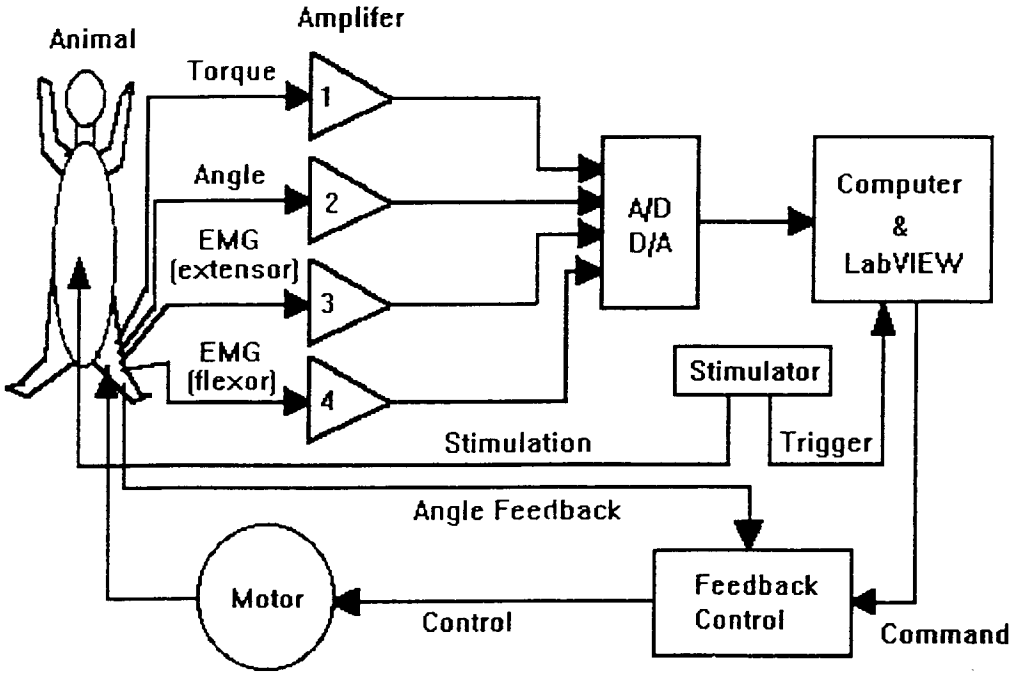


Figure 6